



## ORIGINAL ARTICLE

# Phenotypic Assays to Determine Virulence Factors of Uropathogenic *Escherichia coli* (UPEC) Isolates and their Correlation with Antibiotic Resistance Pattern

Mohsen Tabasi<sup>a</sup>, Mohammad Reza Asadi Karam<sup>a,\*</sup>, Mehri Habibi<sup>a</sup>,  
Mir Saeed Yekaninejad<sup>b</sup>, Saeid Bouzari<sup>a,\*</sup>

<sup>a</sup>Department of Molecular Biology, Pasteur Institute of Iran, Tehran, Iran.

<sup>b</sup>Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.

Received: January 20, 2015  
Revised: August 2, 2015  
Accepted: August 6, 2015

**KEYWORDS:**

antibiotic resistance,  
patients' profiles,  
phenotypic virulence  
traits,  
urinary tract infection,  
uropathogenic  
*Escherichia coli*

**Abstract**

**Objectives:** Urinary tract infection caused by uropathogenic *Escherichia coli* (UPEC) strains is one of the most important infections in the world. UPEC encode widespread virulence factors closely related with pathogenesis of the bacteria. The purpose of this study was to evaluate the presence of different phenotypic virulence markers in UPEC isolates and determine their correlation with antibiotic resistance pattern.

**Methods:** UPEC isolates from patients with different clinical symptoms of UTI were collected and screened for biofilm and hemolysin production, mannose resistant, and mannose sensitive hemagglutination (MRHA and MSHA, respectively). In addition, antimicrobial resistance pattern and ESBL-producing isolates were recorded.

**Results:** Of the 156 UPEC isolates, biofilm and hemolysin formation was seen in 133 (85.3%) and 53 (34%) isolates, respectively. Moreover, 98 (62.8%) and 58 (37.2%) isolates showed the presence of Types 1 fimbriae (MSHA) and P fimbriae (MRHA), respectively. Our results also showed a relationship between biofilm formation in UPEC isolated from acute cystitis patients and recurrent UTI cases. Occurrence of UTI was dramatically correlated with the patients' profiles. We observed that the difference in antimicrobial susceptibilities of the biofilm and nonbiofilm former isolates was statistically significant. The UPEC isolates showed the highest resistance to ampicillin, tetracycline, amoxicillin, and cotrimoxazole. Moreover, 26.9% of isolates were ESBL producers.

\*Corresponding authors.

E-mail: [m\\_asadi12@yahoo.com](mailto:m_asadi12@yahoo.com) (M.R. Asadi Karam), [saeidbouzari@yahoo.com](mailto:saeidbouzari@yahoo.com) (S. Bouzari).

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**Conclusion:** This study indicated that there is a relationship between the phenotypic virulence traits of the UPEC isolates, patients' profiles, and antibiotic resistance. Detection of the phenotypic virulence factors could help to improve understanding of pathogenesis of UPEC isolates and better medical intervention.

## 1. Introduction

Urinary tract infections (UTIs) including cystitis and pyelonephritis are considered to be the second most common type of infections in humans. They account for ~150–250 million cases globally per year [1,2]. More than 50% of women will have at least one episode of UTI during their lifetime [3,4]. Furthermore, after an initial episode of UTI, women are more likely (20–40%) to get recurrent UTIs [4,5]. UTI patients are classified as either asymptomatic or symptomatic cases. Asymptomatic bacteriuria (ABU) occurs in a small number of healthy individuals and may not need treatment, which makes it different from symptomatic bacteriuria. It often affects pregnant women, with varying prevalence among different populations, depending on factors such as age, sex, sexual activity, and the presence of genitourinary abnormalities [3,4,6]. Uropathogenic *Escherichia coli* (UPEC) is the most common cause of urinary tract infections (UTIs) both in community and hospital settings with significant morbidity and mortality worldwide [7–9]. Previous investigations have shown that UPEC strains encode widespread virulence factors closely related to colonization, persistence, and pathogenesis of bacteria in the urinary tract [8,10]. The most important of these factors include adhesins or fimbriae, biofilm formation, and toxins such as hemolysin [8,10]. Fimbriae are categorized serologically by their hemagglutination pattern and receptor specificities as mannose sensitive (MSHA) or mannose resistance hemagglutination (MRHA) [7,11]. Despite the vast subclass of adhesins that have been reported in UPEC, Type I (MSHA) and P (MRHA) are the most common fimbriae found in UPEC strains. They play an important role in binding and invasion to bladder (cystitis) and kidney (pyelonephritis) epithelial cells [12,13].

Biofilm of UPEC provides a nutrient-rich environment which promotes growth and persistence of microorganisms at the site of infection, and protects bacteria from antimicrobial substances [1,14]. Moreover, UPEC strains often express and secrete a labile pore-forming toxin known as  $\alpha$ -hemolysin production that is mainly associated with more virulent UPEC strains [15,16].

Emergence of drug resistance to broad-spectrum beta lactams mediated by extended spectrum beta lactamases (ESBLs) and especially multi-drug resistant (MDR) clonal groups among UPEC strains increase the serious threat to global public health [1,17]. Therefore, to

optimize the use of effective antibiotics for appropriate treatment of UTI patients, it is important for physicians to be aware of the etiological agents and antimicrobial resistance trends of UTI pathogens in their geographic area.

This investigation is aimed to determine the prevalence and correlation of phenotypic virulence traits and antibiotic resistance profile among the UPEC isolated from UTIs, with regard to patients' profiles in Tehran, Iran.

## 2. Materials and methods

### 2.1. Organism collection and identification

The present study was conducted in the Department of Molecular Biology, Pasteur Institute of Iran, Tehran, Iran. Random samples of 156 clinical isolates of UPEC were collected from urine samples of symptomatic and asymptomatic cases of UTIs with significant counts ( $\geq 10^5$  CFU/mL) in various hospitals of Tehran, Iran between March 2013 and February 2014. Only patients aged  $\geq 20$  years who were not on antimicrobial therapy at sample collection or had not taken antibiotic drugs 1 month prior to sampling time, were included in this study. Isolated organisms were identified and characterized on the basis of standard microbiological tests such as Gram staining, catalase, indole, methyl red, voges-proskauer, citrate utilization, motility, fermentation, and utilization of glucose, lactose, and sucrose. All isolates were suspended in 15% glycerol-supplemented Luria-Bertani (LB) medium and preserved frozen at  $-80^\circ\text{C}$ .

### 2.2. Patient demographics

Clinical symptoms, infection history, treatment summary, and all of the necessary relevant information from patients were obtained from hospital records, laboratory reports, and interviewing patients.

### 2.3. Biofilm formation assay

We analyzed the ability of the UPEC isolates to produce the biofilm according to the protocols described by O'Toole and Kolter [18] and Dusane et al [19]. We used the *E. coli* ATCC 25922 strain as a positive control. Briefly, UPEC isolates were grown in LB broth at  $37^\circ\text{C}$  overnight, then the cultures were diluted 1:100 with Tryptic Soy Broth (Merck, Darmstadt, Germany) and incubated in a 96-well polyvinyl chloride (PVC) microtiter plate at room temperature (RT) for 48 hours.

Wells were washed thoroughly with double-distilled water (DDW) and 150  $\mu$ L of 0.1 % (w/v) aqueous solution of crystal violet was added to each well. After 15–20 minutes of incubation at RT, the excess crystal violet dye was washed three times with DDW. Then, 200  $\mu$ L of 33% acetic acid was added to each well and absorbance was measured at optical density 590 nm (OD<sub>590</sub>) with an enzyme-linked immunosorbent assay (ELISA) reader. Each assay was performed in triplicate and the mean values of crystal violet absorbance  $\pm$  SD was calculated for all repetitions of the experiments.

#### 2.4. Hemolysin production

The ability of the UPEC isolates to induce hemolysis on blood agar (Merck) was evaluated to detect the hemolysin producer isolates. The bacteria were inoculated into 5% sheep blood agar and incubated overnight at 37°C. Hemolysin production was detected by the presence of a complete clearing zone of the erythrocytes around the colonies.

#### 2.5. Hemagglutination assay (HA)

UPEC isolates were screened for MRHA and MSHA by using 96-well round bottom plates as described by Hultgren et al [20] and Snyder et al [21]. Approximately, 10<sup>9</sup> CFU/mL of the UPEC bacteria was serially diluted in phosphate-buffered saline (PBS; pH 7.4) and bacterial suspensions were standardized at OD<sub>600</sub> nm of 1.0 and added to the wells. An equal volume of 3% (v/v) solution of guinea pig erythrocytes was added to each well with or without 2% D-mannose (Sigma, St. Louis, MO, USA) and gently mixed with the bacterial suspensions. After incubation at RT for 10 minutes, the hemagglutination results were observed according to the clumping of erythrocytes. Hemagglutination was designated as MRHA when it occurred in the presence of D-mannose and MSHA when it was inhibited by D-mannose. Wells with only the suspension of erythrocytes with or without D-mannose served as negative control and *E. coli* ATCC 25922 was used as a positive control for MRHA.

#### 2.6. Antimicrobial susceptibility patterns

Antibiotic susceptibility patterns of 20 traditional and conventional antibiotics against UPEC isolates was interpreted as recommended by the Clinical and Laboratory Standards Institute (CLSI) by the disk-diffusion method [22]. The following antibiotics (Mast, Co., Merseyside, UK) were included in this study: piperacillin (100  $\mu$ g), imipenem (10  $\mu$ g), meropenem (10  $\mu$ g), piperacillin–tazobactam (100/10 $\mu$ g), ampicillin–sulbactam (10/10  $\mu$ g), amikacin (30  $\mu$ g), cotrimoxazole (25  $\mu$ g), ciprofloxacin (5  $\mu$ g), ceftazidime (30  $\mu$ g), nitrofurantoin (300  $\mu$ g), gentamicin (10  $\mu$ g), ceftriaxone (30  $\mu$ g), nalidixic Acid (30  $\mu$ g), norfloxacin (10  $\mu$ g), cefotaxime (30  $\mu$ g), cefixime (5  $\mu$ g), cefepime (30  $\mu$ g), amoxicillin (25  $\mu$ g), tetracycline (30  $\mu$ g), and ampicillin (10  $\mu$ g). Inhibition zone diameter (mm) of

each antimicrobial disc was measured, and the isolates were classified as resistant and susceptible. The *E. coli* ATCC 25922 was used as control strain.

#### 2.7. Detection of ESBL producers

All of the UPEC isolates that were resistant to third-generation cephalosporins (3GCs) by disc diffusion test, ceftazidime (zone diameter of  $\leq$  22 mm), ceftriaxone (zone diameter of  $\leq$  25 mm), or cefotaxime (zone diameter of  $\leq$  27 mm) were selected for confirmation of ESBL production by double disk synergy test (DDST) as described by CLSI guidelines [22]. *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as nonESBL and ESBL producing organisms, respectively.

#### 2.8. Statistical analysis

Data analysis was performed by using SPSS software version 19.0 for windows (IBM, Chicago, IL, USA). Chi-square test, independent sample *t* test, odd ratio (OR) analysis, two-tailed Fisher's exact tests, and one-way ANOVA analysis test were used to compare the occurrence of phenotypic markers in UPEC and describe the associations of the potential virulence factors with other described factors. A *p* value < 0.05 was considered as statistical significant.

### 3. Results

#### 3.1. Prevalence of UTI in relationship with profiles of patients

Of the 156 UTI patients, the rate of UTI in females (79.5%) was higher than males (20.5%). The incidence of UTI in female patients with age group of 31–40 years (45.2%) and 51–60 years (29.8%) was highest while the age group 41–50 years had the lowest incidence of UTI (8.9%). Among male patients, the highest prevalence of UTI was observed in the age group of 51–60 years (46.9%) and the lowest was seen in the age group of 31–40 years (12.5%). The prevalence of UTI in relation to other important patients' profiles is shown in Table 1. Our results showed that occurrence of UTI were dramatically correlated with the socioeconomic status, educational level, and sexual activity (Table 1).

#### 3.2. Virulence characteristics of UPEC isolates

##### 3.2.1. Biofilm formation

On the basis of our investigations, biofilm formation capacity of all UPEC isolates was classified into four groups, strong biofilm producers (17.3%), moderate biofilm producers (18.6%), weakly biofilm producers (49.4%), and nonbiofilm producers (14.7%).

##### 3.2.2. Hemolysin production

According to our results, hemolysin production was observed in 34% of the UPEC isolates. The remaining 66% isolates showed no hemolysis.

**Table 1.** Prevalence of urinary tract infection in relation to significant patients' profiles.

Patients' profiles		% Urinary tract infection
Socioeconomic status	Well	23.7
	< Intermediate	76.3
Educational level	High	19.9
	< Intermediate	80.1
Patient status	Outpatient (community acquired UTI)	34.6
	Inpatient (hospital acquired UTI)	65.4
Treatment course	Complete	53.8
	Incomplete	46.2
Past history of recurrent	Present	31.4
	Absent	68.6
Sexual activity	Active	74.4
	Not active	25.6
Clinical diagnosis	Acute cystitis	66
	Acute pyelonephritis	14.1
	Asymptomatic bacteriuria	19.9

UTI = urinary tract infection.

### 3.2.3. Prevalence of Type 1 (MSHA) and P fimbriae (MRHA)

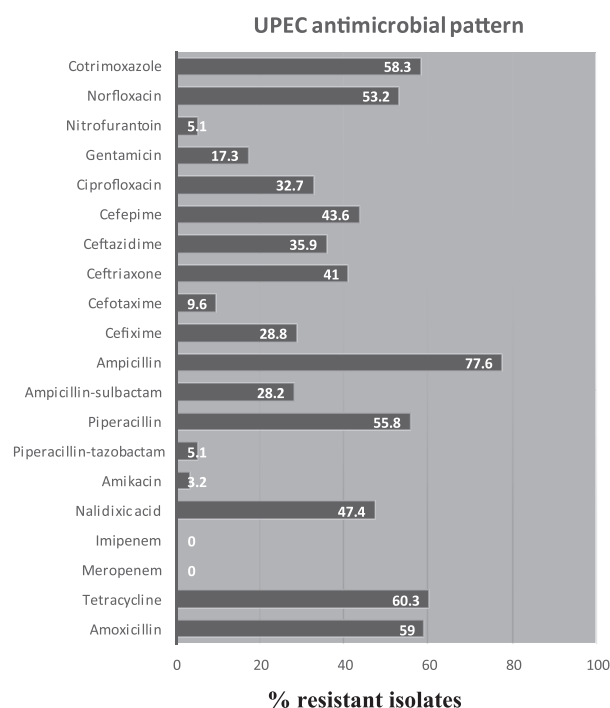
The ability of UPEC isolates to cause agglutination of erythrocytes is an indirect evidence of the presence of fimbriae [23]. In the present study, 62.8% and 37.2% isolates showed the presence of Type 1 (MSHA) and P fimbriae (MRHA), respectively.

### 3.3. Antimicrobial resistance profile of UPEC

The resistance pattern of UPEC isolates to the different antimicrobial agents is shown in Fig. 1. Among the antibiotics tested, ampicillin resistance prevalence was the highest (77.6%), followed by tetracycline (60.3%), amoxicillin (59%), cotrimoxazole (58.3%), and piperacillin (55.8%). The isolates showed the highest sensitivity to antibiotics such as imipenem and meropenem (100%), amikacin (96.8%), piperacillin/tazobactam, and nitrofurantoin (94.9%). Double disc synergy test (DDST) showed that 26.9% of the UPEC isolates were positive for ESBL production. Totally, 123 isolates tested (79%) were multidrug-resistant (isolates with resistance to three or more different classes of antibiotics).

### 3.4. Relation of antimicrobial resistance with virulence determinants of UPEC and patients' profiles

According to our results, overall resistance rates of the antibiotics tested among the UPEC isolated from

**Figure 1.** Antimicrobial resistance pattern of UPEC isolates from patients with urinary tract infections (UTI). UPEC = uropathogenic *Escherichia coli*.

male patients were higher than those of female patients ( $p = 0.04$ ), although the differences in distribution of antimicrobial resistance in different age groups of UTI patients were not statistically significant ( $p > 0.05$ ). We also found that overall resistance rates were higher among inpatients isolates compared to outpatients ( $p = 0.039$ ). Among the isolates, resistance to ampicillin, cefepime, and ceftazidime were significantly more prevalent in inpatients than outpatients ( $p = 0.037$ ,  $p = 0.008$ , and  $p = 0.047$ , respectively). There was also a significant correlation between higher levels of resistance to multiple antibiotics such as nalidixic acid ( $p = 0.038$ ), ceftriaxone ( $p = 0.041$ ), cotrimoxazole ( $p = 0.029$ ), and acute cystitis patients compared with the pyelonephritis and asymptomatic bacteriuria patients (Table 2).

In addition, our results showed that there was no significant difference between resistance to the antibiotics, other than cotrimoxazole ( $p = 0.015$ ), and history of recurrent in the UTI patients. Relationship between antimicrobial resistance and phenotypic virulence markers of the UPEC isolates is shown in Table 3. As shown in Table 3, there was a significant correlation between intensity of biofilm formation and resistance to antibiotics such as ampicillin, norfloxacin, and cotrimoxazole ( $p < 0.05$ ). Moreover, hemolysin producing isolates were significantly less resistant to tetracycline, nalidixic acid, and cotrimoxazole than in nonhemolysin isolates ( $p < 0.05$ ). No relationship was found between

**Table 2.** Antibiotic resistance profile among the urinary tract infection (UTI) patients with different clinical symptoms.

Antibiotics	Acute cystitis %R	Acute pyelonephritis %R	ABU %R	<i>p</i>
AMO	68.9	50	32.2	NS
TET	66	63.6	38.7	NS
NAL	60.2	18.2	25.8	0.038*
PIP	63.1	40.9	41.9	NS
AMP	86.5	59.1	61.3	NS
CRO	51.4	22.7	19.3	0.041*
CAZ	40.8	50	9.7	NS
CPM	50.5	31.8	29	NS
NOR	58.2	54.5	35.5	NS
COT	71.8	45.4	22.6	0.029*

\* Significant at  $p < 0.05$ . ABU = asymptomatic bacteriuria; AMO = amoxicillin; AMP = ampicillin; CAZ = ceftazidime; COT = cotrimoxazole; CPM = cefepime; CRO = ceftriaxone; NAL = nalidixic acid; NOR = norfloxacin; NS = not significant; PIP = piperacillin; TET = tetracycline; %R = percentage of resistance to antimicrobial agents.

MRHA, MSHA isolates, and resistance to the antibiotics used ( $p > 0.05$ ). As shown in Fig. 2, although all of the antibiotic resistance occurred at a higher rate among ESBL producer isolates than nonESBL producer isolates, only resistance to nalidixic acid, piperacillin, ampicillin, and cotrimoxazole was statistically significant ( $p < 0.05$ ).

### 3.5. Phenotypic virulence patterns of UPEC isolates in relation to clinical symptoms

Based on the distribution of the phenotypic virulence traits, all of the UPEC isolates exhibited 16 virulence patterns, referred to as UPEC patterns (UP) in Table 4. The majority of the isolates shared some similarities in most virulence markers, but several differences were also observed. UP 12 was characterized by the presence of the biofilm and MSHA phenotypic markers only, and

was the most common pattern found in 46 isolates. Moreover, some of the UPEC patterns were only observed in single type of clinical symptoms in UTI patients (Table 4). Occurrence of multiple urovirulence markers (isolates with three or more virulence markers) observed in 58 (37.2%) of the UPEC isolates (Table 4). It has been observed that pyelonephritis and cystitis cases were more associated with multiple urovirulence markers compared to asymptomatic bacteriuria cases ( $p = 0.022$  and  $p = 0.038$ , respectively; Table 4).

## 4. Discussion

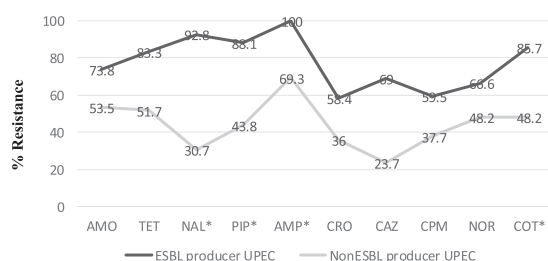
Phenotypic characteristics of UPEC isolates and their correlation with antibiotic resistance patterns in patients with UTI are not well known and few data have been

**Table 3.** Relationship between antimicrobial resistance and virulence factors of UPEC isolates.

Virulence markers		Antimicrobial agents									
		AMO %R	TET %R	NAL %R	PIP %R	AMP %R	CRO %R	CAZ %R	CPM %R	NOR %R	COT %R
Biofilm formation	Strong	82.1	67.8	17.8	71.4	96.4	64.3	60.7	67.8	75	85.7
	Moderate	62	55.2	37.9	58.6	86.2	51.7	44.8	58.6	62.1	69
	Weakly	57.1	66.2	53.2	51.9	75.3	31.2	27.3	31.2	48	50.6
	Negative	31.8	36.4	77.3	45.4	50	31.8	22.7	36.4	31.8	36.4
	<i>p</i>	NS	NS	0.043*	NS	0.042*	NS	NS	NS	0.047*	0.011*
Hemolysin production	Positive	54.7	41.5	9.4	62.3	71.7	39.6	54.7	41.5	52.8	35.8
	Negative	61.2	69.9	67	52.4	80.6	41.7	26.2	44.7	53.4	69.9
	<i>p</i>	NS	0.046*	0.011*	NS	NS	NS	NS	NS	NS	0.043*
HA type	MRHA	53.4	50	60.3	34.5	67.2	43.1	32.1	55.2	46.5	43.1
	MSHA	62.2	66.3	39.8	68.4	83.7	39.8	38.8	36.7	57.1	67.3
	<i>p</i>	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

\* Significant at  $p < 0.05$ . AMO = amoxicillin; AMP = ampicillin; CAZ = ceftazidime; COT = cotrimoxazole; CPM = cefepime; CRO = ceftriaxone; HA = hemagglutination; MRHA = mannose resistant hemagglutination; MSHA = mannose sensitive hemagglutination; NAL = nalidixic acid; NOR = norfloxacin; NS = not significant; PIP = piperacillin; TET = tetracycline; UPEC = uropathogenic *Escherichia coli*; %R = percentage of resistance to antimicrobial agents.





**Figure 2.** Comparison of antimicrobial resistance pattern among the ESBL and nonESBL producer UPEC isolates. \* = significant. AMO = amoxicillin; AMP = ampicillin; CAZ = ceftazidime; COT = cotrimoxazole; CPM = cefepime; CRO = ceftriaxone; ESBL = extended spectrum beta lactamases; NAL = nalidixic acid; NOR = norfloxacin; PIP = piperacillin; TET = tetracycline; UPEC = uropathogenic *Escherichia coli*.

reported in Iran. Identification of virulence markers of UPEC will help to understand their contribution with the antimicrobial resistance [1,2].

Our findings in accordance with other studies indicated that females have a notable frequency of UTI versus males [3,24]. This difference in frequency could be due to several clinical factors, including anatomic differences, hormonal effects, and behavioral patterns [3,24]. Moreover, our investigations reveal that overall resistance rates of antimicrobial drugs were higher in men than women ( $p = 0.04$ ). These observations were similar to the findings of Yilmaz et al [25]. In the current study, most recurrent UTIs in women presented as acute cystitis, as occurred in nonrecurrent women.

We observed that resistance rates were higher among antimicrobials that have been used for a long time as

empirical therapy such as ampicillin and cotrimoxazole. Similar findings were reported in Iran and other countries [2,26]. This may be due to increased consumption of these antibiotics, self-medication, and transfer of resistant isolates [27]. Thus, the use of other antibiotics such as nitrofurantoin and imipenem is recommended for treatment of UTI patients.

Our results showed that the percentage of nalidixic acid resistant isolates causing acute cystitis is more than those causing acute pyelonephritis and asymptomatic bacteriuria ( $p = 0.038$ ). A similar observation was reported by Velasco et al [28]. These data suggest that the nalidixic acid resistant UPEC isolates may have lost the ability to attach the kidney epithelial cells. Moreover, UPEC isolates that produced hemolysin or biofilm were less resistant to nalidixic acid than in nonhemolysis ( $p = 0.011$ ) and nonbiofilm formers ( $p = 0.043$ ). These findings in accordance with other studies suggest that resistance to nalidixic acid may be associated with marked reduction in the virulence severity of UPEC isolates [29–31]. Also, this is in accordance with the observation of Johnson et al [32] that UPEC strains with greater antibiotic resistance tended to express lower virulence traits.

The present study showed that hemolysin producer isolates had a higher ability of biofilm formation. This finding is similar to the study of Soto et al [29] and in contrary to observations of Marhova et al [33].

According to our results, some urovirulence markers were closely associated with a specific anatomical site of infection. In this regard, we observed that hemolysin production is more frequent in UPEC isolated from patients with pyelonephritis than in isolates from patients with other clinical symptoms of UTI ( $p = 0.01$ ). This finding reveals that hemolysin may be contributing

**Table 4.** Distribution of virulence patterns of UPEC in relationship with clinical symptoms.

Patterns	Phenotypic markers					No. of isolates	Clinical symptoms		
	ESBL	Biofilm	Hemolysin	MRHA	MSHA		Acute cystitis	Acute pyelonephritis	Asymptomatic bacteriuria
UP 1	+	+	+	+	–	9	3	6	0
UP 2	+	+	+	–	+	13	7	4	2
UP 3	+	+	–	+	–	5	4	0	1
UP 4	+	+	–	–	+	9	7	0	2
UP 5	+	–	+	+	–	1	0	1	0
UP 6	+	–	+	–	+	1	1	0	0
UP 7	+	–	–	+	–	2	1	0	1
UP 8	+	–	–	–	+	2	0	0	2
UP 9	–	+	+	+	–	6	2	3	1
UP 10	–	+	+	–	+	14	8	3	3
UP 11	–	+	–	+	–	31	22	2	7
UP 12	–	+	–	–	+	46	37	1	8
UP 13	–	–	+	+	–	3	1	2	0
UP 14	–	–	+	–	+	6	6	0	0
UP 15	–	–	–	+	–	1	0	0	1
UP 16	–	–	–	–	+	7	4	0	3

ESBL = extended spectrum beta lactamases; MRHA = mannose resistant hemagglutination; MSHA = mannose sensitive hemagglutination; UP = UPEC pattern; UPEC = uropathogenic *Escherichia coli*.

to the severity of UPEC infections. Moreover, our results showed a higher potency of biofilm production among UPEC isolates causing acute cystitis than isolates from acute pyelonephritis and, in particular, those causing asymptomatic bacteriuria ( $p = 0.04$ ). In fact, biofilm formation seems to be one of the most important virulence factors among the UPEC isolated from patients with acute cystitis. Also, these biofilm producing UPEC isolates showed a significantly greater expression of Type 1 fimbriae (MSHA) than nonbiofilm producing isolates ( $p = 0.021$ ). This could be explained by the important role of Type 1 fimbriae in the first steps of biofilm formation [12]. One-way analysis of variance indicated that the difference in antimicrobial susceptibilities of the biofilm and nonbiofilm producing isolates was statistically significant ( $p < 0.05$ ). Murugan et al [34] have reported the correlation between biofilm formation and multiple drug resistance among UPEC strains. We also found a remarkable correlation between biofilm and ESBL producing UPEC.

In this study, production of MRHA and MSHA was in relation to clinical symptoms of UTI patients. MRHA isolates were significantly higher among the UPEC isolated from pyelonephritis patients ( $p = 0.025$ ), whereas MSHA isolates were more prevalent in patients with cystitis and asymptomatic bacteriuria ( $p < 0.05$ ). Association of P fimbriae (MRSA) of UPEC with acute pyelonephritis in UTI patients suggests that P fimbriae are required for colonization and invasion of the human upper urinary tract [35,36].

Our results showed that UPEC isolated from patients with symptomatic bacteriuria are characterized by higher virulence characteristics than those isolated from patients with asymptomatic UTI (Table 4). This observation reflects the important role of virulence factors of UPEC in severity of clinical symptoms of UTI patients.

In our study, biofilm producing UPEC isolates were strongly linked to recurrent UTI ( $p = 0.001$ ). These data suggest that the tropism of UPEC isolates for the recurrent infections may depend on the biofilm formation.

In conclusion, the present study provides novel epidemiological information relevant to the UPEC isolated from UTI patients. These findings indicate that there is a correlation between the most important phenotypic virulence traits of the UPEC isolates and antibiotic resistance. Also, we believe that detection of the phenotypic virulence factors could be valuable in investigations on the pathogenesis of UPEC isolates and management of UTI therapy. However, further studies of genotypic and phenotypic characteristics of UPEC isolates may help us to get novel insights into pathogenesis of UPEC isolates.

## Conflicts of interest

There is no conflict of interest.

## Acknowledgments

This article is part of the work by Mohsen Tabasi to fulfill the requirement for a Master of Science. This work was financially supported by Pasteur Institute of Iran.

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